A Possible Vitamin A_2

THE antimony trichloride colour test for vitamin A is associated with a maximum absorption at 620 mμ. We have, since 1929, repeatedly encountered an additional band near 693 mμ, but further characterization of the chromogen has been hindered by the presence of excessive amounts of vitamin A. Thus, in halibut liver oils the relative intensities 620 mμ/693 mμ are *c.* 6 : 1, and in halibut visceral oils 10 : 1. The 693 mμ chromogen is rarely detectable in cod liver oils and never in our experience in whale liver oils.

It is frequently absent from the vitamin A fraction of eyes, but one of us (J.R.E.) has observed the 693 mμ band in extracts from goldfish eyes, the ratio 620 mμ/693 mμ being *c.* 1 : 1·5. Experiments on brown trout have shown that the 693 mμ chromogen occurs in the non-saponifiable extracts from livers and viscera. The 620 mμ band, as such, could not be detected. Direct absorption spectra showed the presence of three broad bands with maxima at 470, 350 and 287 mμ, respectively, the ultra-violet bands varying in intensity with the 693 mμ band in the colour test. Lederer and Rosanova[1] have also found an intense band at 693 mμ in the colour test applied to freshwater fish liver oils from the neighbourhood of the Murmansk Sea. We understand (private communication) that an apparent connexion between absorption bands at *c.* 345 and 285 mμ and the 693 mμ chromogen has been confirmed by Prof. Heilbron and Dr. Gillam on Lederer's oils. Wald's discovery that a substance apparently identical with the 693 mμ chromogen can replace the vitamin A of rhodopsin *without loss of physiological function* runs parallel with the similar replacement in the viscera and liver of brown trout. It therefore seems desirable provisionally to designate as 'vitamin A_2' the 693 mμ chromogen with its characteristic ultra-violet absorption.

In chemical separations with liver oils, the 693 mμ chromogen follows vitamin A, the ratio 620 mμ/693 mμ for a given species remaining very nearly constant. It is not difficult with some liver oil extracts to reach $E^{1\%}_{1cm.}$ 693 mμ 500–1,000, but judging from the amount of vitamin A present, the pure substance will have $E^{1\%}_{1cm.}$ 693 mμ $\lessapprox$ 5,000. On this basis, each brown trout contains of the order 0·12 mgm. of the new material.

It seems clear that the 693 mμ chromogen is not in any simple sense an artefact derived from vitamin A; but the position remains obscure with regard to bands at 640 mμ and 660 mμ which occasionally appear in the colour test.

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[1] *Biochimica*, **2**, 293 (1937).

Specificity of Indophenol in the Estimation of Ascorbic Acid in Fermented Products

DURING the course of an investigation on the antiscorbutic value of Kaffir beer, it was observed that beers treated with a 2 per cent concentration of metaphosphoric acid gave high values for 'vitamin C' when titrated with indophenol in the usual manner, the values varying between 1·1 and 38·5 mgm./100 ml.

Delf[1], working with guinea pigs and monkeys, concluded that Kaffir beer was of slight antiscorbutic value. Levy and Fox[2], using hydrochloric acid for acidification, examined eleven specimens of mine-brewed beer and found values of 0·2–0·5 mgm./100 ml.

Bernhauer *et al.*[3] showed that when *Aspergillus niger* was allowed to grow in sugar mixtures, substances with the same reducing properties as ascorbic acid were formed. Whether these possessed antiscorbutic activity or not was not clear (Křiženecký and Nevalonnyj[4] and Hermann and Fodor[5]).

As with ascorbic acid, norite charcoal (Fox and Levy[6]) completely removed the reducing power. However, only a small fraction (0·8 mgm. out of 13·0 mgm.) was restored after hydrogen sulphide treatment. Hubbard squash extract (Tauber *et al.*[7]) entirely removed the reducing power. The folin uric acid reagent (Medes[8]) gave identical values with indophenol titration in the presence of 2 per cent metaphosphoric acid.

The effect of *p*H on the indophenol titration of the beer was as follows:

INDOPHENOL REDUCING POWER OF KAFFIR BEER AT DIFFERENT *p*H VALUES.
(in mgm. 'ascorbic acid' per 100 ml. beer).

2·5 *N* HCl	8·0
1·2 *pH*	0·8
1·4	0·8
1·6	0·8
1·8	0·8
2·0	(doubtful end-point) 1·5
2·2	13·0
2·4	13·0
2·6	13·0

Guinea pig experiments showed the reducing substance to be almost devoid of antiscorbutic activity, the animals dying of scurvy in three to four weeks. Controls on orange juice remained healthy, and on autopsy showed no signs of scurvy.

The reducing power of the beer was followed at different stages of its preparation, and it was found that a large increase took place during the boiling of the mash, and a further increase during fermentation.

From these experiments it is concluded that the bulk of the reducing substance is not ascorbic acid. The true ascorbic acid content of the beer probably corresponds to the values of about 0·8 mgm./100 ml. obtained with the norite procedure and by titration at a *p*H between 1·2 and 1·8. The concentration was far too low to have any appreciable effect on the guinea pigs in the doses given. It is also shown that ascorbic acid oxidase (cf. Zilva[9]) and Srinivasan[10]), and the folin uric acid reagent (cf. Fujita *et al.*[11]) are not specific for ascorbic acid.

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[1] Delf, *Publ. S. Afr. Inst. Med. Res.*, **2** (No. 14), 47 (1921).
[2] Levy and Fox, *S. Afr. Med. J.*, **9**, 181 (1935).
[3] Bernhauer, Görlich and Köcher, *Biochem. Z.*, **286**, 60 (1936).
[4] Křiženecký and Nevalonnyj, *Z. Untersuch. Nahr. u. Genussm.*, **66**, 278 (1933). (Quoted by Bernhauer *et al.* (3).)
[5] Hermann and Fodor, *Biochem. Z.*, **276**, 323 (1935).
[6] Fox and Levy, *Biochem. J.*, **30**, 208 (1936).
[7] Tauber, Kleiner and Mishkind, *J. Biol. Chem.*, **110**, 211 (1935).
[8] Medes, *Biochem. J.*, **29**, 2251 (1935).
[9] Zilva, *Biochem. J.*, **30**, 1215 (1936).
[10] Srinivasan, *Biochem. J.*, **30**, 2077 (1936).
[11] Fujita, Akiji and Ebihara, *Biochem. Z.*, **290**, 182, 192 (1937).